REMARKS

The above amendment along with the following remarks are being submitted as a full and complete response to the Official Action dated march 18, 2003, the period for response to which is set to expire June 18, 2003.

Claims 1-4 and 6-14 are under consideration in this application. Claim 5 is being cancelled without prejudice or disclaimer. Claims 1-4 and 6-14 are amended, as set forth above and in the attached marked-up presentation of the claim amendments, in order to more particularly define and distinctly claim applicants' invention. Applicants hereby submit that no new matter is being introduced into the application through the submission of this response.

In view of the above amendments and the following remarks, the Examiner is respectfully requested to give due reconsideration to this application, to indicate the allowability of the claims, and to pass this case to issue.

Formality Rejection

Claims 3-10 were rejected under 35 U.S.C. § 112, second paragraph, as being vague and indefinite. As indicated, the claims are being amended for clarification. Accordingly, the withdrawal of the outstanding informality rejection is in order, and is therefore respectfully solicited.

Prior Art Rejection

Under 35 U.S.C. § 102(b), claims 1-2 were rejected as being anticipated by WO 98/28440 to Nyren (hereinafter "Nyren"), and claims 1-8 were rejected as being anticipated by U.S. Pat. No. 4,971,903 to Hyman (hereinafter "Hyman"). Under 35 U.S.C. § 103(a), claims 9-13 were rejected as being unpatentable over Hyman in view of Nyren, and claim 14 was rejected over Hyman in view of Nyren further in view of U.S. Pat. No. 5,795,762 to Abramson et al. (hereinafter "Abramson"). These rejections have been carefully considered, but are most respectfully traversed.

The method of analysis of DNA sequence according to the invention is characterized in (1) a step of removing or inactivating the pyrophosphates and/or the apyrase in the reagent after

the degrading step in claim 1 or the adding step in claims 3-4, or (2) a step of removing or inactivating the pyrophosphate in each of the solutions after the first step in claims 11, 12. By removing the impurities apyrase or pyrophosphates, or inactivating them by adding inhibitors for them, the apyrase and pyrophosphates are prevented from generating noise which disturbs the measurement in the subsequent detecting step (page 19, lines 5-9; page 20, lines 12-18; page 25, lines 5-8). For instance, PPase and/or apyrase are immobilized to a solid, e.g. beads, magnetic beads or the like, which facilitates the addition or removal of PPase and/or apyrase from each reagent solution (page 9, lines 4-8).

Applicants contend that neither Nyren nor Hyman teaches or suggests such a step of removing or inactivating the pyrophosphates and/or the apyrase from a reagent solution after a degrading step thereby preventing them from generating measurement noises in the subsequent pyrophosphoric acid detecting step.

In contrast, Nyren merely identifies a base at a target position in a sample DNA sequence by detecting pyrophosphate (Ppi) enzymically (Abstract). The sequencing procedure is carried out with simultaneous degradation of nucleotides by apyrase. However, Nyren does NOT concern or suggest the removing or inactivating of pyrophosphates and/or the apyrase in a reagent solution after a degrading step so as to improve the efficiency of the subsequent pyrophosphoric acid detecting step.

Hyman provides a method for sequencing nucleic acid polymer. If appropriate nucleotides are provided, the production of the complementary polymer occurs to release Ppi. The sequence of the complementary polymer and thus the template polymer is determined by analyzing the presence of Ppi (Abstract). Specifically, a pyrophosphates column is used to ***remove*** "**contaminating Ppi**" ("*The PPase enzyme is very potent and only small quantities are needed to remove essentially all of the PPi from a sample*" col. 3, lines 9-12, 40-42, 48-50) rather than ***remove*** or inactivate the "**pyrophosphates and/or the apyrase**" in a reagent solution after a degrading step so as to improve the efficiency of the subsequent pyrophosphoric acid detecting step. Although Hyman teaches in claims 8-9 a step of "*treating the feedstock to remove* ***inorganic pyrophosphate***", such a removing step occurs "*prior to providing the feedstock to the polymerization environment* (claim 8)" "*in which production of the complementary polymer will occur if appropriate nucleotides are provided* (Abstract lines 5-7)"such that the removing step happens prior to (rather than "after") a degrading step and a pyrophosphoric acid detecting step.

Abramson fails to compensate for Nyren or Hyman's deficiencies. Abramson merely shows thermostable DNA polymerases which exhibit a different level of 5' to 3' exonuclease activity than their respective native polymerases (Abstract).

Accordingly, the present invention as now recited in all the claims is distinguishable and thereby allowable over the rejections raised in the Office Action. The withdrawal of the outstanding prior art rejections is in order, and is respectfully solicited.

In view of all the above, clear and distinct differences as discussed exist between the present invention as now claimed and the prior art reference upon which the rejections in the Office Action rely, Applicant respectfully contends that the prior art references cannot anticipate the present invention or render the present invention obvious. Rather, the present invention as a whole is distinguishable, and thereby allowable over the prior art.

Favorable reconsideration of this application is respectfully solicited. Should there be any outstanding issues requiring discussion that would further the prosecution and allowance of the above-captioned application, the Examiner is invited to contact the Applicant's undersigned representative at the address and phone number indicated below.

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Marked-up Version of Amended Claims

1. A method of analysis of DNA sequence, [which comprises] comprising the steps of:

degrading, by pyrophosphates, pyrophosphoric acid contained in a reagent used for extension reaction of a DNA primer hybridized to a target nucleic acid through a complementary [strand] binding, and/or degrading, by apyrase, adenosine 5'-triphosphate contained in the reagent;

removing or inactivating the pyrophosphates and/or the apyrase in the reagent after the degrading step;

conducting the extension reaction[,]; and

detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step.

2. A method of analysis of DNA sequence according to Claim 1, wherein the pyrophosphates and/or the apyrase [has been] is immobilized on a solid.

3. A method of analysis of DNA sequence, [which comprises] comprising steps of:

adding pyrophosphates to one or more solutions [which contain] each containing a different deoxynucleotide[s, respectively], or [to one or more solutions which contain] an analogue of the [different] deoxynucleotide[s, respectively, at least one of which is an analogue thereof,] thereby degrading pyrophosphoric acid contained in the solutions[, and];

removing or inactivating the pyrophosphates and/or the apyrase in the solutions after the step of degrading after the adding step;

extending a DNA primer[, which has been] hybridized to a target nucleic acid via a complementary [strand] binding, by using [the DNA primer,] DNA polymerase and at least one of the solutions [obtained in said step]; and

detecting pyrophosphoric acid [thus] generated [by] during an [the] extension reaction by chemiluminescence-reaction after the removing or inactivating step.

4. A method of analysis of DNA sequence[, which comprises] comprising steps of:

adding pyrophosphates to one or more solutions [which contain] each containing a different deoxynucleotide[s, respectively], or [to one or more solutions which contain]

an analogue of the [different] deoxynucleotide[s, respectively, at least one of which is an analogue thereof,] thereby degrading pyrophosphoric acid contained in the solutions[, and];

removing or inactivating the pyrophosphates and/or the apyrase in the solutions after the step of degrading after the adding step;

extending a DNA primer [, which has been] hybridized to a target nucleic acid via a complementary [strand] binding, by using [the DNA primer,] DNA polymerase and at least one of the solutions [obtained in said step,] and converting pyrophosphoric acid, [thus] generated [by] during the extension reaction, into adenosine 5'-triphosphate in [the] presence of adenosine 5'-phosphosulfate and ATP sulfurylase[,]; and

detecting luminescence caused by chemiluminescence-reaction [containing] using the adenosine 5'-triphosphate, a luminescence-enzyme and a luminescence substrate after the removing or inactivating step.

6. A method of analysis of DNA sequence according to Claim 4, wherein [the first step] the step of adding the pyrophosphates compromises a step of adding the pyrophosphates to at least one of the solutions containing the DNA-primer [-containing solution], the DNA-polymerase [-containing solution], the luminescence-enzyme [-containing solution], the luminescence-substrate [-containing solution], the adenosine 5' – phosphosulfate[-containing solution and], or the ATP-sulfurylase [-containing solution], thereby degrading the pyrophosphoric acid contained therein [at least one of said solutions], and/or adding apyrase to degrade at least one of the solutions containing the adenosine 5' – phosphosulfate [contained in at least one of said solution].

7. A method of analysis of DNA sequence according to Claim 6, further comprising a step of removing or inactivating the pyrophosphates and/or the apyrase [contained] added in [the pyrophosphates-and/or] said at least one of the solutions [apyrase -added solution].

8. A method of analysis of DNA sequence according to Claim 7, wherein the pyrophosphates and/or the apyrase [has been] is immobilized on a solid.

9. A method of analysis of DNA sequence according to Claim 4, wherein [the] a base at the

3' terminus of the primer is complementary to [the] one base [one base behind the 3' terminus site of single nucleotide polymorphism of] located next to a single nucleotide polymorphism at one side of a 3' terminus in the target nucleic acid.

10. A method of analysis of DNA sequence according to Claim 4, wherein [the] a second or third base from the 3'_terminus of the DNA primer [has been] is substituted with a base not complementary to [the] one base sequence of the target nucleic acid.

11. A method of analysis of DNA sequence, [which comprises] comprising steps of:

a first step of adding pyrophosphates to each of a solution containing deoxyadenosine 5'-α-thiotriphosphate, a solution containing deoxythymidine 5'-triphosphate, a solution containing deoxyguanosine 5'-triphosphate and a solution containing deoxycytidine 5'-triphosphate, thereby degrading pyrophosphoric acid contained in each of the solutions;

a second step of removing or inactivating the pyrophosphates in each of the solutions[, and];

a third step of extending a DNA primer[, which has been] hybridized to a target nucleic acid via a complementary [strand] binding, by using [the DNA primer,] DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid [thus] generated [by] during the extension reaction into adenosine 5'-triphosphate in [the] presence of adenosine 5'-phosphosulfate and ATP sulfurylase[,]; and

a fourth step of detecting luminescence caused by chemiluminescence-reaction [containing] using the adenosine 5'-triphosphate, luciferase and luciferin after the second step.

12. A method of analysis of DNA sequence, [which comprises] comprising steps of:

a first step of adding pyrophosphates to a solution containing deoxyadenosine 5'-α-thiotriphosphate, deoxythymidine 5'-triphosphate, deoxyguanosine 5'-triphosphate and deoxycytidine 5'-triphosphate, thereby degrading the pyrophosphoric acid contained in the solution;

a second step of removing or inactivating the pyrophosphates in each of the

solutions after the first step[, and];

a third step of extending a DNA primer[, which has been] hybridized to a target nucleic acid via a complementary [strand] binding, by using [the DNA primer,] DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid, [thus] generated [by] during the extension reaction, into adenosine 5'-triphosphate in [the] presence of adenosine 5'-phosphosulfate and ATP sulfurylase[,]; and

a fourth step of detecting luminescence caused by chemiluminescence-reaction [containing] using the adenosine 5'-triphosphate, luciferase and luciferin after the second step.

13. A method of analysis of DNA sequence according to Claim 12, wherein [the] a second or third base from the 3' terminus of the DNA primer [has been] is substituted [by] with a base not complementary to [the] one base sequence of the target nucleic acid.

14. A method of analysis of DNA sequence according to Claim 12, wherein the extension reaction is conducted by [degrading the strand, which has been extended by the extension reaction, from the 5' terminus thereof by the 5' -> 3' exonuclease reaction and] repeating [complementary strand] hybridization of the DNA primer to the target nucleic acid via degrading extended a strand produced in the extension reaction from the 5' terminus of the extended strand, using a 5' -> 3' exonuclease reaction.